jc715 U.S. PTO
11/16/00

PATENT
Customer Number 22,852
Attorney Docket No. 02356.0077-01

jc862 U.S. PTO
09/713017
11/16/00

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

ASSISTANT COMMISSIONER FOR PATENTS
Washington, D.C. 20231

Prior Application: Art Unit: 1635
Examiner: J. Epps

SIR:
This is a request for filing a **Continuation Application under 37 C.F.R. § 1.53(b)** of pending prior application Serial No. 09/000,227 filed May 11, 1998 of André CHOULIKA et al., for NATURAL OR SYNTHETIC RETROELEMENT SEQUENCE ENABLING NUCLEOTIDE SEQUENCE INSERTION INTO A EUKARYOTIC CELL.

1. ☒ Enclosed is a complete copy of the prior application including the oath or Declaration and drawings as originally filed. I hereby verify that the attached papers are a true copy of prior application Serial No. 09/000,227 as originally filed on May 11, 1998 (French with Verified English Translation).

2. ☐ Enclosed is a substitute specification under 37 C.F.R. § 1.125.

3. ☒ Cancel Claims 2-24.

4. ☒ A Preliminary Amendment is enclosed adding new claims 25-40.

5. ☒ The filing fee is calculated on the basis of the claims existing in the prior application as amended at 3 and 4 above.

Basic Application Filing Fee					$710	$ $710.00
	Number of Claims		Basic	Extra Claims		
Total Claims	22	-	20	2	x $18	36.00
Independent Claims	2	-	3	0	x $80	-0-
☒ Presentation of Multiple Dep. Claims					+$270	270.00
Subtotal						$ 1,016.00
Reduction by 1/2 if small entity						- -0-
TOTAL APPLICATION FILING FEE						$ 1,016.00

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6. ☒ A check in the amount of **$1,016.00** is enclosed (to cover the filing fee, additional claims fee and multiple dependent claims fee).

7. ☒ The Commissioner is hereby authorized to charge any fees which may be required including fees due under 37 C.F.R. § 1.16 and any other fees due under 37 C.F.R. § 1.17, or credit any overpayment during the pendency of this application to Deposit Account No. 06-0916.

8. ☒ Amend the specification by inserting before the first line, the sentence:

-- This is a continuation of application Serial No. 09/000,227, filed May 11, 1998, which is incorporated herein by reference. --

9. ☐ New formal drawings are enclosed.

10. ☒ The prior application is assigned of record to: **Institut Pasteur of Paris, France**

11. ☒ Priority of application Serial No. PCT/FR96/01178, filed on July 25, 1996 in France is claimed under 35 U.S.C. § 119. A certified copy
☒ is on file in the prior application.

12. ☐ A verified statement claiming small entity status

☐ is enclosed or ☐ is on file in the prior application.

13. ☒ The power of attorney in the prior application is to at least one of the following: FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER, L.L.P., Douglas B. Henderson, Reg. No. 20,291; Ford F. Farabow, Jr., Reg. No. 20,630; Arthur S. Garrett, Reg. No. 20,338; Donald R. Dunner, Reg. No. 19,073; Brian G. Brunsvold, Reg. No. 22,593; Tipton D. Jennings, IV, Reg. No. 20,645; Jerry D. Voight, Reg. No. 23,020; Laurence R. Hefter, Reg. No. 20,827; Kenneth E. Payne, Reg. No. 23,098; Herbert H. Mintz, Reg. No. 26,691; C. Larry O'Rourke, Reg. No. 26,014; Albert J. Santorelli, Reg. No. 22,610; Michael C. Elmer, Reg. No. 25,857; Richard H. Smith, Reg. No. 20,609; Stephen L. Peterson, Reg. No. 26,325; John M. Romary, Reg. No. 26,331; Bruce C. Zotter, Reg. No. 27,680; Dennis P. O'Reilley, Reg. No. 27,932; Allen M. Sokal, Reg. No. 26,695; Robert D. Bajefsky, Reg. No. 25,387; Richard L. Stroup, Reg. No. 28,478; David W. Hill, Reg. No. 28,220; Thomas L. Irving, Reg. No. 28,619; Charles E. Lipsey, Reg. No. 28,165; Thomas W. Winland, Reg. No. 27,605; Basil J. Lewris, Reg. No. 28,818; Martin I. Fuchs, Reg. No. 28,508; E. Robert Yoches, Reg. No. 30,120; Barry W. Graham, Reg. No. 29,924; Susan Haberman Griffen, Reg. No. 30,907; Richard B. Racine, Reg. No. 30,415; Thomas H. Jenkins, Reg. No. 30,857; Robert E. Converse, Jr., Reg. No. 27,432; Clair X. Mullen, Jr., Reg. No. 20,348; Christopher P. Foley, Reg. No. 31,354; John C. Paul, Reg. No. 30,413; David M. Kelly, Reg. No. 30,953; Kenneth J. Meyers, Reg. No. 25,146; Carol P. Einaudi, Reg. No. 32,220; Walter Y. Boyd, Jr., Reg. No. 31,738; Steven M. Anzalone, Reg. No. 32,095; Jean B. Fordis, Reg. No. 32,984; Roger D. Taylor, Reg. 28,992; Barbara C. McCurdy, Reg. No. 32,120; James K. Hammond, Reg. No. 31,964; Richard V. Burgujian, Reg. No. 31,744; J. Michael Jakes, Reg. No. 32,824; Thomas W. Banks, Reg. No. 32,719; Christopher P. Isaac, Reg. No. 32,616; Bryan C. Diner, Reg. No. 32,409; M. Paul Barker, Reg. No. 32,013; Andrew Chanho Sonu, Reg. No. 33,457; David S. Forman, Reg. No. 33,694; Vincent P. Kovalick, Reg. No. 32,867; James W.

SCANNED, # 18

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14. ☒ The power appears in the original declaration of the prior application.

15. ☐ Since the power does not appear in the original declaration, a copy of the power in the prior application is enclosed.

16. ☒ Please address all correspondence to FINNEGAN, HENDERSON, FARABOW, GARRETT and DUNNER, L.L.P., 1300 I Street, N.W., Washington, D.C. 20005-3315, **Customer Number 22,852**.

17. ☐ Recognize as associate attorney ______________________

18. ☐ Also enclosed is ______________________

PETITION FOR EXTENSION. If any extension of time is necessary for the filing of this application, including any extension in the parent application, serial no. 09/000,227, filed May 11, 1998, for the purpose of maintaining copendency between the parent application and this application, and such extension has not otherwise been requested, such an extension is hereby requested, and the Commissioner is authorized to charge necessary fees for such an extension to our Deposit Account No. 06-0916. A duplicate copy of this paper is enclosed for use in charging the deposit account.

FINNEGAN, HENDERSON, FARABOW, GARRETT & DUNNER, L.L.P.

Dated: November 16, 2000

By: Kenneth J. Meyers

Kenneth J. Meyers
Reg. No. 25,146

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PATENT
Attorney Docket No. 02356.0077-01

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: André CHOULIKA et al.	
Serial No.: NEW	Group Art Unit: Not yet Assigned
Filed: November 16, 2000	Examiner: Not yet Assigned
For: NATURAL OR SYNTHETIC RETROELEMENT SEQUENCE ENABLING NUCLEOTIDE SEQUENCE INSERTION INTO A EUKARYOTIC CELL	

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

PRELIMINARY AMENDMENT

Prior to the examination of the above application, please amend this application as follows:

IN THE TITLE:

Please amend the title to read -- Retroelement sequence enabling nucleotide sequence insertion into a eukaryotic cell and elimination of unnecessary proviral sequences --.

ABSTRACT:

Please add the Abstract provided on the attached page.

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IN THE CLAIMS:

Please cancel claims 2-24. Please add the following new claims 25-40:

-- 25. A nucleic acid molecule comprising

retroelements that comprise a recombinant provirus when a target cell is infected by a retrovirus containing said retroelements;

a nucleotide sequence of interest, which can be expressed in the target cell and which can be transferred with said retroelements into the target cell and integrated into the recombinant provirus; and

a recognition sequence for the elimination of proviral sequences in the recombinant provirus, which are not necessary for expression of the nucleotide sequence of interest in the target cell after integration of the recombinant provirus into the target cell.

26. The nucleic acid molecule as claimed in Claim 25, wherein said retroelements comprise cis-acting region and the nucleotide sequence of interest is incorporated into the cis-acting region.

27. The nucleic acid molecule as claimed in Claim 25, wherein said retroelements comprise a 3' LTR or a 5' LTR region and the nucleotide sequence of interest is incorporated into the 3' LTR or 5' LTR region.

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28. The nucleic acid molecule as claimed in Claim 25, wherein the retroelements comprise a U3 region of a 3' LTR, a U5 region of a 5' LTR, and an R region, and the nucleotide sequence of interest is incorporated into one of said regions.

29. The nucleic acid molecule as claimed in Claim 25, wherein the recognition sequence is recognized by a recombinase.

30. The nucleic acid molecule as claimed in Claim 29, wherein the recognition sequence, which can be recognized by a recombinase, is situated upstream or downstream from the nucleotide sequence of interest.

31. The nucleic acid molecule as claimed in Claim 25, wherein the recognition sequence is located upstream or downstream from the nucleotide sequence of interest.

32. The nucleic acid molecule as claimed in Claim 25, wherein said nucleic acid molecule comprises a nucleotide sequence coding for a recombinase that recognizes said recognition site.

33. The nucleic acid molecule as claimed in Claim 32, wherein the retroelements comprise 5' LTR and 3' LTR regions and the nucleotide sequence coding for the recombinase is situated between the 5' LTR and 3' LTR regions.

34. The nucleic acid molecule as claimed in Claim 32, wherein the nucleotide sequence coding for the recombinase encodes CRE protein.

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35. The nucleic acid molecule as claimed in Claim 33, wherein the nucleotide sequence coding for the recombinase encodes CRE protein.

36. The nucleic acid molecule as claimed in any one of Claims 33, 34, or 35, wherein the recognition sequence comprises a LoxP recognition site.

37. The nucleic acid molecule as claimed in Claim 25, wherein the nucleotide sequence of interest encodes a polypeptide or RNA.

38. The nucleic acid molecule as claimed in Claim 37, wherein the RNA is antisense RNA or a ribozyme sequence.

39. The nucleic acid molecule as claimed in Claim 25, which is contained in a plasmid deposited under C.N.C.M. Assession No. I-1599.

40. A retroviral vector comprising a nucleic acid molecule according to any one of Claims 25-29.--

REMARKS

New claims 25-40 were originally prosecuted and cancelled without prejudice or disclaimer, in co-pending parent Application Serial No. 09/000,227.

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If there is any fee due in connection with the filing of this Preliminary Amendment, please charge the fee to our Deposit Account No. 06-0916.

Respectfully submitted,

FINNEGAN, HENDERSON, FARABOW,
GARRETT & DUNNER, L.L.P.

Dated: November 16, 2000

By: Kenneth J. Meyers
Reg. No. 25,146

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ABSTRACT

A nucleic acid molecule is provided comprising 1) retroelements comprising a recombinant provirus when a target cell is infected by a retrovirus containing the retroelements; 2)a nucleotide sequence of interest, which can be transferred into the target cell and integrated into the recombinant provirus; and 3) a recognition sequence for the elimination of proviral sequences in the recombinant provirus. Proviral sequences that are not necessary for expression of the nucleotide sequence of interest in the target cell are eliminated once the recombinant provirus integrates into the target cell.

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EX834A.

VERIFICATION OF A TRANSLATION
(VERIFICATION D'UNE TRADUCTION)

I, the below named translator, hereby declare that :

My name and post office address are as stated below :

That I am knowledgeable in the English language and in the language in which the below identified international application was filed, and that I believe the English translation of the international application n° PCT/FR96/01178 is a true and complete translation of the above identified international application as filed.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true ; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of the application or any patent issued thereon.

Date 21 janvier 1998

Full name of the translator JARVIS, DEREK
(Nom et prénon du traducteur)

Signature of the translator Derek Jarvis
(Signature du traducteur)

Post Office Address (Adresse postale) 7, rue d'UPSAL
67000 Strasbourg

SEQUENCE OF NATURAL OR SYNTHETIC RETROELEMENTS ENABLING NUCLEOTIDE SEQUENCE INSERTION INTO A EUKARYOTIC CELL

The present invention relates to a sequence of natural or synthetic retroelements, in particular an LTR retroviral nucleotide sequence, more particularly retroviral DNA as well as a retroviral vector containing this sequence and which enables, on infection of a cell into which it is desired to integrate a gene of interest contained in this vector, a large part of the proviral sequences which are no longer necessary after integration of the recombinant provirus to be eliminated.

Recent progress in the use of retroviruses as gene vectors falls into three classes : (i) improvement of the packaging cell lines, (ii) manipulation of the tropism of the envelope proteins, (iii) expression of multiple genes. The modification of the structure of the integrated provirus has not yet been very much exploited because this latter contains certain cis-acting essential elements. These elements are the source of many problems.

From the point of view of the structure, the retroviral vector system is constructed on two elements : the transcomplementing genes (gag, pol and env) and the cis-acting sequences (U3, R, U5, the polypurine track (PPT), the primer binding site (PBS) and the packaging and dimerization signal).

The transcomplementing genes are incorporated in transcomplementing cells. They are not transferred to the target cells.

The cis-acting sequences are incorporated into retroviral vectors. Most of them are transferred to the infected target cells and are integrated into the recombinant provirus. The elimination of any one of these cis-acting sequences results in a non-functional retroviral system. The packaging signal is necessary for the packaging of the retroviral genome in viral particles. PBS, PPT and R are required for reverse transcription. The U3 and U5 sequences are essential for reverse transcription and for the integration of the retroviral product introduced into the cell.

Once the provirus is integrated, these sequences are no longer necessary for the expression of the gene. On the contrary, these sequences may even cause many problems. (i) A transcriptional interference may result from the presence of the strong promoter in the U3 sequences. (ii) The PBS may function as a cis-acting inhibitory

element for the internal promoters. For example, in the multipotent cells, the PBS is a recognition sequence for a strong repressor and in addition it acts as an inhibitory element. (iii) The U3 sequence contains at least one of the negative regulatory elements situated in the direct repeated activators at a distance between -345 and -306. (iv) The 3' LTR (long terminal repeat) can activate genes flanking cells with sometimes deleterious consequences for the cell. (v) The RNA expressed from the promoter of the 5' LTR contains the packaging signal and can, consequently, be recovered in a retroviral particle. This RNA can recombine with a cellular RNA or the retroviral RNA of a phenotype, resulting in the recovery of a retrovirus endowed with novel properties (creating a potential biological danger). These problems have not yet been entirely overcome since all these elements are necessary for the replication and the insertion of the virus into the genome.

In the context of the present invention, the inventors have developed a sequence of natural or synthetic retroelements, in particular a retroviral nucleotide sequence, and in particular a retroviral vector containing this sequence, designed so as to permit the elimination of a large part of the proviral sequences which are no longer necessary after integration of the provirus into a cell host. More particularly, this retroviral sequence is a retroviral DNA capable of permitting the integration of a single LTR sequence, a retrotransposon or a sequence comprising a U3, R or U5 region into the genome of a cell host.

The invention also relates to host cells, preferably eukaryotic cells, obtained after transfection by the retroelement sequence of the invention or a vector containing it.

The present invention hence concerns a retroelement sequence characterized in that it comprises an insertion sequence incorporated in a region capable of being transferred into a target cell and integrated into a recombinant provirus when this target cell is infected by a retrovirus containing this sequence. In particular, this insertion sequence is incorporated into a cis-acting region, more particularly in the 3' LTR or the 5' LTR region and preferably in the U3 region of the 3' LTR or the U5 region of the 5' LTR of this retroviral sequence. This insertion sequence comprises a nucleotide sequence of interest capable of being integrated into the genome of a cell host as well as a recognition sequence for a recombinase. Preferably, all of the retroviral sequence only contains a single recognition sequence situated with the sequence of interest, upstream or downstream for example, although this latter parameter is

not absolutely necessary. The nucleotide sequence of interest may be situated downstream from the recognition sequence in as much as this latter is included in the region transferred into a target cell on infection of this target cell by a retrovirus containing the retroviral sequence. The maximal length of the insertion sequence that the retroviral vector of the invention contains is usually situated between 0.5 and 10 kb.

In particular, the sequence of the invention also comprises a DNA sequence coding for a recombinase capable of recognizing the sequence of its recognition site, this DNA sequence coding for a recombinase being advantageously situated between the 5' LTR and the 3' LTR regions of the vector. The invention also relates to a retroviral vector or recombinant retrovirus comprising the sequence described above. Preferably, the retroviral vector, which may take the form of a recombinant retrovirus, only contains a single recognition sequence incorporated into the region capable of being transferred to a target cell.

The DNA of the invention hence makes possible the insertion, through the intervention of a retroviral vector, of nucleotide sequences of interest in host cells, for example of the eukaryotic type, without the insertion of the proviral sequences which are no longer necessary after the integration of the sequences of interest in the provirus.

The term "nucleotide sequence of interest" used above refers to sequences to be inserted in the genome of host cells in order to allow these latter to produce molecules of interest, more particularly in therapy or for vaccination. These sequences of interest include, among others, genes, DNA or RNA sequences coding either for proteins (hormones, immunoglobulins, enzymes or others) when these retroviral vectors of the invention are used in gene therapy, or human or non-human proteins (such as viral proteins) when the retroviral vectors of the invention are to be used in the framework of vaccination protocols. The nucleotide sequences of interest may also be constituted in part of regulatory elements (i.e. promoter, enhancer) homologous or heterologous to the host cell on the one hand and, on the other hand, of sequences coding for all or part of one or more genes or complementary DNA. Furthermore, the nucleotide sequences of interest may also code for an antisense RNA or a ribozyme sequence.

The possible applications of the retroelement sequence of the present invention are manifold. The sequence of the invention is used either simply for the insertion of nucleotide sequences in host cells such as eukaryotic cells in an environment which promotes a better expression

of this gene, or in gene therapy, or in vaccination. As an example, the sequence of interest described in Nature Medicine, Volume No. 7, July 1995 corresponding to the insert of the plasmids $pMEPV_H/pMEPV_L$ or $pMEPV_H/pREPV_K$ can be used and the in vivo expression product may be an element of a therapeutic composition.

The retroviral vector of the invention is obtained by the transfection of a transcomplementing viral cell line with a retroelement sequence of the invention existing preferably in the form of a plasmid and comprising preferably in one of its LTRs and more particularly in its right LTR a nucleotide sequence of interest as well as a recognition sequence. The plasmid may also contain a nucleotide sequence coding for a recombinase capable of recognizing the recognition site situated upstream or downstream from this sequence of interest, this nucleotide sequence being advantageously situated between the 3' LTR and 5' LTR regions. Once the transfection of the viral cell line has been performed, a retroviral vector is obtained which can be used for the infection of a cell host in which it is desired to integrate the nucleotide of interest.

The plasmid defined above as well as the method of transfection of the cell line by said plasmid are also included in the framework of the present invention.

The invention also relates to a method or procedure for the introduction of nucleotide sequences of interest into a cell host such as a eukaryotic cell. The method is characterized in that it comprises the introduction of a sequence corresponding to retroelements such as the U3, U5 and/or R regions into a eukaryotic cell, said sequence being linked to a nucleotide sequence of interest. This method is characterized by an infection step of a eukaryotic cell by a retroviral vector containing the retroviral sequence of the invention which contains the nucleotide sequence to be introduced into the eukaryotic cell under conditions permitting the integration into the cell genome of the cell of a single LTR of the retroviral vector containing the sequence to be introduced in the genome of the host cell and a second step of expression of the sequence of interest then the elements of the product of this latter starting from the recombinant cell.

The invention also relates to a method of medical treatment comprising the administration of an effective amount of a retroviral vector of the invention including a nucleotide sequence containing a gene capable of being integrated into the genome of certain target cells of the

patient. The retroviral vectors of the present invention can thus be used in gene therapy.

The invention also relates to a cell host which has integrated into its genome a proviral structural containing a single LTR region of a retrovirus. This LTR sequence contains a single copy of a nucleotide sequence of interest. More particularly, the cell host of the invention is a eukaryotic cell and the proviral structure is characterized in that it is essentially free of the PBS sequence, the packaging and dimerization signal and/or the PPT signal.

The invention also relates to the recombinant cell hosts containing the unique LTR sequence or a part of this latter and the sequence of interest.

The invention also relates to the use of the retroelement sequence or the retroviral vector of the invention in the production in host cells of proteins encoded in a nucleotide sequence of interest included in this retroviral DNA or in the medical treatment of a patient for the purpose of integrating a nucleotide sequence of interest in the genome of target cells.

More particularly, the sequence of the invention also makes possible the transfer of nucleotide sequences coding for antibodies into host cells. One speaks of extracellular immunization since the antibodies generated from these sequences produce their effect within the cells which have integrated them. Among the nucleotide sequences coding for antibodies which can be integrated into host cells by using the retroviral sequence and the retroviral vector of the invention, mention may be made as non-limiting examples of the antibodies recognizing envelope proteins of the HIV viruses, particularly the gp 120 proteins of the HIV-1 virus and the HIV-2 virus as well as antibodies making it possible to block the reverse transcriptase. Such antibodies are described in the publication by Maciejewski et al. (Nature Medicine, volume No.7, July 1995) and elsewhere. Since the retroviral vector of the invention makes possible a more efficacious integration of these nucleotide sequences of interest, the therapeutic efficacy of this approach is all the more improved.

The invention hence relates to the use of retroviral vectors comprising a nucleotide sequence of interest coding for an antibody recognizing a HIV viral protein or protein fragment, as well as a recognition nucleotide sequence, these two nucleotide sequences being situated in a region which can be transferred into human lymphocytes

subsequent to an infection. Preferably, these two sequences are situated in the 3' LTR region of the retroviral vector.

The invention also relates to an expression method or procedure for a molecule encoded by a nucleotide sequence of interest in a host cell. The method comprises :

- the infection of the host cell by a retroviral vector of the invention containing the nucleotide sequence of interest;
- the growth of the host cell under conditions permitting the expression of the nucleotide sequence of interest; and
- the production of the desired molecule.

The retroviral nucleotide sequence as well as the retroviral vectors of the invention can be used in several ways to enable the desired nucleotide sequence of interest to be integrated. In fact, the inventors have demonstrated that it was not necessary to associate the expression of the recombinase directly with the retroviral vector, although the integration yields are higher when a nucleotide sequence coding for the recombinase is an integral part of the vector.

In a first embodiment of the invention, a retroviral vector containing in the 3' LTR or the 5' LTR region of the retroviral vector a first nucleotide sequence comprising a nucleotide sequence of interest as well as a recognition sequence situated downstream or upstream from the sequence of interest, as well as a second nucleotide sequence identical with the first nucleotide sequence inserted in the 5' LTR region of the vector, is introduced into the genome of a host cell by infection. A plasmid comprising a nucleotide sequence coding for a recombinase capable of recognizing the recognition sequence is also introduced into the cell by transfection. The expression of the recombinase by transfection transforms the structure of a provirus with two LTRs into a provirus with a single LTR. In this type of reaction, about 50% of the stable transformants contain the modified provirus. This partial result may be explained by the ephemeral expression of the plasmid comprising the nucleotide sequence coding for the recombinase after the transfection, resulting in recombination without integration.

In a second embodiment of the invention, use is made of a retroviral sequence containing a nucleotide sequence containing a sequence of interest as well as a recognition sequence situated downstream or upstream from this sequence of interest. The complete sequence is inserted in the 3' LTR region of a retrovirus. The retroviral vector thus obtained is then used to infect a cell line which expresses

constitutively or by induction a recombinase capable of recognizing the recognition sequence. The efficacy of such a system is high, especially when the gene coding for the recombinase is expressed right from the start of the retroviral infection.

In a third embodiment of the invention, a retroviral vector comprises a nucleotide sequence inserted into its 3' LTR region. This nucleotide sequence contains a sequence of interest as well as a recognition sequence which can be recognized by a recombinase. The recognition sequence is situated downstream or upstream from the sequence of interest. Furthermore, a gene coding for a recombinase as well as a promoter are integrated into the retroviral vector between the two LTRs of this vector. A cell line is then infected with this retrovirus. Such a retroviral vector only generates proviruses with a single LTR. The efficacy of this system is high since all of the integrated and analyzed events have this structure. The principal advantage of such a retroviral vector is that it is associated with successful recombination events between the two LTR with the deletion of the gene coding for the recombinase. Consequently, the infected cells only express the recombinase ephemerally.

In one of the preferred embodiments of the invention, the inventors used the Crelox recombinase system specific for the site of the bacteriophage P1. The recognition site LoxP was inserted into the 3' LTR upstream from the nucleotide sequence of interest in a ΔEnh type vector, and the gene coding for the recombinase *cre* between the two LTRs. In the producing cell line, the Cre protein is expressed from the proviral plasmid construction. However, since the plasmid only contains a single target loxP, it is not recombined by the Cre protein. After infection, the loxP site is duplicated from the 3' LTR into the 5' LTR. Thus, Cre recombines the two direct repeated loxP sites, which results in the deletion of all the sequences included between the two loxP, including the PBS, the dimerization and encapsidation signal and the PPT. Thus only a single LTR containing the reporter gene remains integrated in the cell genome. The *cre* gene is also lost during recombination.

In all the preferred embodiments described above, the nucleotide sequence of interest, which is generally a sequence foreign to the retrovirus, is advantageously introduced into the U3 region of 3' LTR of the retroviral sequence. In fact, the possibility of introducing sequences, even complete transcriptional units, into the U3 region of 3' LTR is very well documented. Nonetheless it is possible to envisage the introduction

of sequences of interest elsewhere than in the U3 region of the 3' LTR of the retroviral vector. Examples include the U5 region of the 5' LTR. Furthermore, although it is preferable that the recognition site is situated immediately downstream or upstream from the nucleotide sequence of interest, it is also possible to place this sequence at an appreciable distance which may vary between 32 nt and 4.5 kb without affecting the final integration of the sequence of interest into the target cell.

The quantity of foreign genetic material introduced into the U3 region of the viral DNA may be high and in some cases higher than 4 kbp. These sequences may even correspond to a complete transcriptional unit with an independent promoter, which demonstrates the versatility of these vectors.

The possibility of introducing considerable quantities of foreign genetic material into the 3' LTR of the retroviral vector of the invention makes this retroviral vector a system of choice for the insertion of gene sequences into cells, more particularly into eukaryotic cells. Several types of eukaryotic cells can thus be transformed. Of the eukaryotic cells which may be used in the context of the present invention, particular mention should be made of the following types of cells : NIH 3T3, BRL, HM1, D3, PLL4, LT.

In one of the particular embodiments of the present invention, provision is made for the insertion of a nucleotide sequence coding for a marker in the 3' LTR region of the retroviral vector. The presence of this marker makes it possible to prove the insertion of the new product.

As non-limiting examples, the following markers may be used : LacZ, GFP (green fluorescent protein), CD9, PAL (alkaline phosphatase) and HRP (Horse Radish Peroxidase).

Although the Crelox recombinase system constitutes a system of choice for the retroviral vector of the invention, other recombinase systems can also be used. As non-limiting examples, the following recombination systems may be used : the yeast FLP system (called "Flip") and the bacterial R system.

The retroviral vectors of the invention hence furnish an efficient agent for the transfer of DNA into host cells. The proviral integration is precise and does not cause chromosomal rearrangement. The design of duplicating vectors set out from the principle that the transposition of the gene into the 5' LTR, outside the retroviral transcriptional unit, improves its expression.

Although the presence of the encapsidation signal makes possible the generation of a virus competent for replication by recombination between the endogenous sequences of the retrovirus and the provirus, that the presence of PPT and other cis-elements make transposition possible and that the presence of the PBS sequences may have negative effects on the expression of the transgene, all these events are very improbable when a retroviral vector according to the invention is used since all these viral sequences are deleted.

The system of the LTRs is a novel concept in the construction of retroelements in general. There are many retroviruses, the host spectrum of the particle of which is of interest but for which the design of vectors requires the presence of certain cis- or trans-acting sequences different from those which have been mentioned previously which make possible a correct assembly of the virion and enable it to be infectious, like for example the human HIV and HTLV viruses or the goat virus CAEV. Once integrated, the cis or trans regulating elements become useless for the transduced gene but have pathogenic effects for the cell and significantly increase the probabilities of the resurgence of the wildtype virus. The necessity of this type of sequence for this type of retrovirus makes them practically unusable for employment as vectors. The application of the LTRs system of the present invention in the case of these retroviruses makes it possible to envisage a usage as vectors for the transduction of genes with more transcriptional safety and fidelity.

The present invention should be clearly described with reference to the following non-limiting examples which make reference to the following figures :

- Figure 1A presents the structure of the plasmid pMloxPL;
- Figure 1B presents the structure of the provirus MloxPL resulting from the infection with the retrovirus MloxPL;
- Figure 1C presents a provirus after the recombination induced by cre using the two loxP sites within the LTRs;
- Figure 1D presents an analysis by "Southern blot" of the cellular DNA derived from NIH3T3 fibroblasts infected by MloxPL;
- Figure 1E presents an analysis by "Southern blot" of the cellular DNA derived from the clone NIH3T3 MloxPL.1 transfected by the plasmid pMC1-Cre;
- Figure 2A presents the structure and molecular analysis of the provirus MloxPL resulting from the infection with the retrovirus

MloxPL and the recombination induced by Cre using the two loxP sites within the LTRs;

- Figure 2B presents the results of an analysis by "Southern blot" of the cellular DNA derived from NIH 3T3 Cre.1 fibroblasts infected by MloxPL with digestion by the restriction endonuclease BclI;

- Figure 2C presents the results of an analysis by "Southern blot" of the cellular DNA derived from NIH 3T3 Cre.1 fibroblasts infected by MloxPL with digestion by the restriction endonuclease EcoRV;

- Figure 3A presents the structure of the plasmid pMCreloxPL;

- Figure 3B presents a diagram of the 3' LTR of several preferred embodiments of the retroviral vector of the invention; - Figure 3C presents the results of an analysis by "Southern blot" of the cellular DNA derived from NIH 3T3 fibroblasts infected by MCreloxPL by digestion by the restriction endonuclease BclI;

- Figure 3D presents the results of an analysis by "Southern blot" of the cellular DNA derived from NIH 3T3 fibroblasts infected by MCreloxPL by digestion by the restriction endonuclease EcoRV;

- Figure 3E presents the results of an analysis by "Southern blot" of the cellular DNA derived from NIH 3T3 fibroblasts infected by MCreloxPL by digestion by the restriction endonuclease KpnI;

- Figure 4 presents a scheme for the mechanism of the generation of LTRs with the retroviral vector McreloxPL.

EXAMPLES

Nomenclature

The nomenclature of the various vectors and different cells was established as follows : p designates the plasmid vector (for example, pMloxPL); the names with a ψ 2 (deposited on 10/3/1993 with the ECAC under the number 93031002) designate the cell line of the wildtype helper virus ψ 2 transfected with this vector (for example, ψ2-MloxPL) ; the names without p designate the viruses produced by the cells of the wildtype helper virus (for example, MloxPL); the name of the cell line followed by the name of the virus indicates that the cell line contains a provirus resulting from an infection (for example, NIH 3T3MloxPL).

Culture and selection of the cells

The established cell lines NIH3T3 (mouse fibroblasts) and ψ2 (encapsidation cell line of the mouse ecotropic retrovirus) are referenced in (7) and (13). They are cultured in a DMEM (Eagle medium modified

by Dulbecco) with a high glucose content (4.5 g/l) supplemented with 5% fetal calf serum. The cells are incubated at 37°C in a moist atmosphere containing 12% of CO_2. G418 is added to the appropriate medium at a concentration of 600 mg/ml. The cloning of colonies resistant to G418 was done by pipetting individual colonies and isolating them in a separate culture dish. The cloning of the infected cells is performed by limiting dilution 48 hours after the infection.

Transfection, infection, staining of the cells and analysis of the nucleic acids by "Southern blot"

Precipitations by means of calcium phosphate were performed as described in (12). The supernatant containing the virus was used to infect NIH 3T3 cells in the presence of 5 µg/ml of polybrene, as previously described in (37). Hybridizations by "Southern blot" were prepared by means of the procedure in reference (6). The β-galactosidase activity was detected by staining with X-gal, as previously described in (38).

Detection of LacZ by PCR

The PCR is performed on 1 µg of genomic cellular DNA in a reaction of 40 µl (50 mM Tris-HCl (pH9), 150 µg/ml of bovine serum albumin, 16 mM $(NH_4)_2SO_4$, 7 mM $MgCl_2$, 250 µM for each dNTP, 1.25 U Taq DNApol (USB), 0.078 U Vent(exo+) DNApol (N.E. Biolabs)). Primers were added at a final concentration of 0.25 µM (for 20 mers). The PCR was heated at 80°C for 5 minutes before the start. 35 cycles (94°C, 55 min.; 59°C, 30 s; 70°C, 3 min. 30 s) with the following primers :

5' - GCATCGAGCTGGGTAATAAGCGTTGGCAAT - 3' and
5' - GACACCAGACCAATGGTAATGGTAGCGAC - 3'
for the detection of LacZ and
5' - GGACTGGGTGGCTTCCAACTCCCAGACAC - 3' and
5' - AGCTTCTCATTGCTGCGCGCCAGGTTCAGG - 3'
for the detection of the endogenous RAP-SYN of mouse, as internal standard. The reaction products of the PCR were analyzed by gel electrophoresis.

Example 1 - Construction of the retroviral vector pMloxPL and transfection of cell lines

a) Construction of the vector pMloxPL

pMloxPL results from the plasmid (described in Choulika et al., 1995) in which the loxP recognition site is inserted within the Nco I site in a linker
(5' CATGCATATAACTTCGTATAGCATACATTATACGAAGTTATC- 3' and 5'- CATGGATAACTTCGTATAATGTATGCTTATCGAAGTTATATG - 3' in place of the I-Sce I site. The sequence of the loxP site was verified by sequencing of the DNA.

The retrovirus used is illustrated in Fig. 1a. pMloxPL is constructed from a defective Moloney murine leukemia provirus (lacking the gag, pol, and env genes) by inserting the sequence coding for PhleoLacZ in the U3 region of the right LTR in the place of the activation sequences at a distance. 5' derived from the PhleoLacZ gene was placed between the splicing acceptor site of the *env* gene of MoMuLV and a 32 bp long loxP site derived from the bacteriophage P1. In the infected cells this type of virus has the LTR which contains the gene enhanced by a flanking cell promoter by trapping of the promoter.

b) Transfection of cell lines with the plasmid pMloxPL

The cell lines producing the virus were generated by transfecting the plasmid pMloxPL with the selection plasmid pUSVneo in the cell line of the wildtype helper virus ψ 2 which expresses the ecotropic wildtype helper virus defective for encapsidation. After selection for G418 (neomycin), individual clones selected for their β-galactosidase activity were tested to titer the virus. The titration was performed by cloning NIH3T3 cells immediately after infection by limiting dilution, and by detecting the presence of the provirus containing LacZ by PCR. The cell lines producing ψ2-MloxPL.1 and ψ2-MloxPL.2 showed a titer on the NIH 3T3 cell line of 2.5 x 10^4 and 5 x 10^4 (Table 1), respectively. The titration of the units forming blue colonies per milliliter (BCFU/ml) of the viral supernatant of ψ2-MloxPL.1 and ψ2-MloxPL.2 is 3 and 6, respectively, which corresponds to an approximate ratio of 1 blue colony per 10^4 integrations. As we have previously described, these proviruses function as promoter traps, and only one integration out of 10^4 expresses the reporter gene in the NIH3T3 fibroblast cell line.

The structures of the DNA of the integrated MloxPL proviruses are schematized in Figure 1b. In the Figures to the present text, the positions of the LTRs and of the BclI restriction sites and the sizes of the fragments are indicated (x indicates the BclI fragment of random size of the left arm and y the BclI fragment of random size of the right arm. P indicates a LacZ probe radiolabelled with ^{32}P. These structures were then analyzed by means of hybridization by "Southern blot" in 6 independent NIH 3T3 clones. The results of this analysis are presented in Figure 1d. All of the clones contain a provirus in which the sequences within the U3 region of the 3' LTR have been duplicated in the 5' LTR; the digestion by the endonuclease BclI generates the expected fragment from the part of the proviruses with two LTR containing PhleoLacZ (the restriction enzyme BclI has a recognition site in each LacZ sequence in the LTRs). The hybridization by "Southern blot" using lacZ as probe, shows a 6 kbp cleavage fragment demonstrating a faithful duplication of the U3 region. The two additional bands of variable sizes represent fragments which extended from the BclI sites in the provirus to the flanking cellular DNA. The two additional bands show that there is one proviral integration per clone, and their variable sizes confirm that each cell line was an independent clone.

Example 2 : Cotransfection of cell lines with the plasmids pMloxPL and pMCl-Cre

We have examined whether the loxP duplication through the intermediary of the LTR may be recombined by the Cre recombinase. We have used the clonal cell line NIH3T3MloxPL.1 to target a recombination with the Cre protein. We have cotransfected into this cell line the expression vector pMC1-Cre with the selection vector pUSVneo. The structure of the provirus after the recombination induced by Cre is illustrated in Figure 1c.

The DNA of 24 clones resistant to neomycin was analyzed by means of hybridization by "Southern blot". The detection of the recombined proviruses was performed by analyzing the BclI digestions. The restricted DNA of the clones was probed with a LacZ radiolabelled with ^{32}P. The results of this analysis are illustrated in Figure 1e. The parental structure of the provirus shows a 6 kbp band corresponding to the faithful duplication of the U3 sequence containing LacZ and two

additional bands of 8 and 2 kbp (described above (Fig. 1b & d)). The analysis of the DNA of the clones resistant to neomycin shows 5 clones out of 24 in which the 6.0 kbp band has been eliminated (Fig. 1e). The bands of 8 and 2 kbp corresponding to the cell DNA flanking the provirus were still present, thus showing that the proviral integration site has not been rearranged. This result suggests that the expression of the *cre* gene in a cell containing the MloxPL provirus may lead to a frequent recombination of the two loxP sites included in the LTR.

In order to test the efficiency of the recombination through the intermediary of Crelox, we have also analyzed the DNA of 25 clones resistant to G418 by means of hybridization by "Southern blot" in order to test the presence of the *cre* sequence. The DNA was digested with the restriction endonuclease XhoI and probed with a full length *cre* DNA radiolabelled with ^{32}P. The results of this analysis are illustrated in Figure 1e. Three of the five clones recombined in loxP show a band hybridizing with the 1.9 kbp *cre* probe suggesting that the full length *cre* expression unit of pMC1-Cre is present. One of the five clones recombined in loxP did not show a band hybridizing with the *cre* probe. One clone recombined in loxP shows a 5 kbp band resulting from a rearrangement of the pMC1-Cre plasmid. Four other clones showing a 1.9 kbp band hybridizing with *cre* do not show recombination in the integrated provirus MloxPL. We have not detected the presence of *cre* DNA in the remaining 16 clones. This result shows that the efficacy of the recombination induced by the presence of the pMC1-Cre is not absolute in the stable transfected cell lines, where only 50% of the loxP sites are recombined. This result also suggests that the recombination between the two loxP sites may be obtained through the ephemeral expression of pMC1-Cre (Table 2).

Example 3 - Infection of the cells expressing the Cre enzyme with MloxPL

We have produced an NIH3T3 cell line containing the pMC1-Cre by cotransfection of the plasmids pMC1-Cre and pUSVneo. Clones were selected in a medium containing G418 and their DNA was analyzed after digestion with the restriction endonuclease XhoI by means of hybridization by "Southern blot". The presence of the Cre gene was detected by hybridization with a full length *cre* DNA probe radiolabelled with ^{32}P. Ten of the 12 clones resistant to G418 analyzed revealed the

presence of the Cre sequence in a variable number of copies (ranging approximately from 1 to 10 copies) (data not shown).

We chose the clone NIH3T3Cre.1 containing approximately 10 copies of the pMC1-Cre plasmid for the purpose of supplementary analysis. We infected the clone NIH3T3Cre.1 with the MloxPL virus. The clones were isolated by limiting dilution.

The structure of the provirus resulting from the infection of the MloxPL retrovirus and the recombination induced by Cre is illustrated in Figure 2a. The structure of the integrated vector is a solitary LTR (sLTR). It lacks an LTR and all of the sequences situated between the two LTRs in the integrated proviruses (Figs. 2a & c).

The structure of the DNA of the MloxPL proviruses was analyzed by means of hybridization by "Southern blot" after digestion by the restriction endonuclease BclI of their cell DNA in order to detect the presence or absence of duplicated LTR in the proviral structure. As demonstrated in Figure 2b, the analysis of the 12 clones resulting from the infection of NIH3T3Cre.1 by MloxPL showed the absence from all of the clones of the 6 kbp band indicative of the duplication.

In order to demonstrate the absence of the 5' LTR from the provirus, a restriction of the DNA was performed with EcoRV. The hybridization by "Southern blot" of the DNA of NIH3T3Cre.1.MloxPL, restricted by the endonuclease EcoRV by using a radiolabelled LacZ probe (Figs. 2a & c), shows the presence of a 2.1 kbp band and a band of random size. The absence of a 3.9 kbp band demonstrates the absence of a 5' LTR.

Example 4 - Construction of the retroviral vector pMcreloxPL and transfection of cell lines

a) Construction of the pMcreloxPL plasmid

The structure of the retrovirus used in the present example is illustrated in Fig. 3a. pMCreloxPL results from the insertion of the 1.3 kbp *cre* gene fused with a nuclear localization of the large T antigen of the simian virus 40 between the two LTR of the pMloxPL plasmid. The *cre* gene is under the transcriptional control of the promoter of the thymidine kinase gene (tk) of the herpès simplex virus flanked by a duplication of the enhancer at a distance of the polyoma mutant virus PYF441 with linkers at the PstI site of pMloxPL. The Cre sequence is in the same

orientation as the viral genome. The pMcreloxPL plasmid was deposited with the CNCM on 13 June 1995 under No.I-1599.

b) Transfection of cell lines with the plasmid pMcreloxPL

Cell lines producing the virus were generated by cotransfecting the pMCreloxPL plasmid with the selection plasmid pUSVneo into the encapsidation cell line ψ 2. After selection of the transfected ψ 2 cells in a medium containing G418, individual clones were selected for the production of a retrovirus expressing LacZ. One clone produced an infectious virus; this clone was called ψ 2-MCreloxPL.1. In ψ 2-MCreloxPL.1, the pMCreloxPL plasmid was integrated as a single copy into the host cell genome (data not shown). The cell lines producing ψ 2-MCreloxPL.1 produce 1 x 10^4 infectious viruses per milliliter; the presence of the LacZ gene is detected by PCR (Table 1). The titration of the blue colony forming units (BCFU/ml) of the ψ 2-MCreloxPL.1 viral supernatant is 3 BCFU/ml, which corresponds as expected to a approximate diminution of 10^4 with respect to the titration by PCR. The production of ψ 2-MCreloxPL was of low efficiency compared with the production of ψ 2-MloxPL, a single producing clone having been recovered after several cotransfection experiments. This clone had integrated a single copy of the viral plasmid construction. We deduced from this that the cells which integrate more than one copy of the plasmid cannot be recovered owing to the presence of the recombinase activity which modified the integrated transgenes.

c) Proviral structure of MCreloxPL

The isolation of NIH3T3 cells infected with MCreloxPL was performed by limiting dilution, after infection at a multiplicity of 0.5 viral particle per cell. The structure of the provirus resulting from the infection of the retrovirus pMCreloxPL is illustrated in Figure 3b. In this figure, α indicates the random size fragment of the left arm.

The structure of the DNA of the integrated proviruses was analyzed by means of hybridization by "Southern blot" in 6 independent NIH3T3MCreloxPL clones. The analysis by "Southern blot" of the proviral DNA restricted by the BclI endonucleases and detected with a radiolabelled LacZ probe generated two fragments of variable sizes, and

these clones did not generate an additional 7.5 kbp band (Figure 3c). The absence of this additional 7.5 kbp fragment demonstrates the presence of a single LTR containing PhleoLacZ. In order to establish in addition that the proviral structure corresponds to a single LTR, an additional analysis was undertaken. In order to demonstrate definitively the absence of a 5' LTR in the provirus, a restriction by EcoRV of the cellular DNA was performed. The hybridization by "Southern blot" of the DNA of NIH3T3MCreloxPL, restricted by the endonuclease EcoRV by using a radiolabelled LacZ probe (Fig. 3d) shows the presence of a 2.1 kbp band and a band of random size. The absence of a 6 kbp band demonstrates the absence of a 5' LTR. This "blot" was then hybridized with a radiolabelled *cre* probe. The absence of a band in the DNA of NIH3T3MCreloxPL shows that the proviral DNA lacked the *cre* gene (Fig. 3d). Finally, in order to establish that the structure of the remaining LTR had not rearranged, the DNA of NIH3T3MCreloxPL was restricted by the endonuclease KpnI and probed with a DNA of radiolabelled LacZ. A 4.3 kbp fragment was observed in all cases, showing that the LTRPhleoLacZ was of the expected size (Fig. 3e).Consequently, all of the isolated clones contained an integrated provirus with a single unrearranged LTR.

TABLE 1. Titers of the cell lines ψ 2-MloxPL and MCreloxPLa

Clone	IP/ml[b] (10^4)	BCF/ml[c]	Ratio[d] (10^{-4})
ψ2-MloxPL.1	2.5	3	1.2
ψ2-MloxPL.2	5	6	1.2
ψ2-MCreloxPL.1	1	3	3

[a]Clones resistant to G418 derived from the clone NIH3T3MloxPL.1 cotransfected with pMC1-Cre and pUSVneo

[b] indicates the presence of the pMC1-Cre sequence
(+) indicates the presence of a correct 1.9 kbp XhoI fragment derived from pMC1-Cre detected by a *cre* probe labelled with ^{32}P.
(*) indicates the presence of a XhoI fragment hybridizing with the ^{32}P radiolabelled *cre* probe, but of incorrect size.

(-) indicates the absence of the pMC1-Cre plasmid.

[c]Results of the loxP recombination through the intermediary of Cre detected by means of "Southern blot" analysis.

TABLE 2. loxP Recombination through the intermediary of a pMC1-Cre transfection

Number of clones resistant to G418[a]	Presence of pMC1-Cre[b]	loxP[c] recombination
3	+	+
1	-	+
1	*	+
4	+	-
16	-	-

[a]$\psi 2$ cells were transfected respectively with either pMloxPL+pUSVneo or pMCREloxPL+pUSVneo, clones resistant to G418 were selected in a medium containing G418. Stocks of virus were prepared by incubating 8 ml of medium with 5×10^6 cells of each clone resistant to G418, 5×10^4 NIH3T3 cells were subjected to various dilutions of the virus for 8 hours and then cloned by limiting dilution or tested for their β-galactosidase activity by staining with X-gal.

[b]The titers were calculated by the ratio between the infected clones isolated after cloning and detected by PCR (IP : infectious particle) and the uninfected clones (PCR+/PCR-)/1 x 10^5)

[c]BCFU is the acronym for "blue colony forming unit". The BCFU are infections resulting in a trapping of the gene which activates the PhleoLacZ detected by staining with X-gal.

[d] The ratio is the number of BCFU per infected clone.

CLAIMS

1. Sequence of synthetic or natural retroelements, in particular of retroviral DNA, characterized in that it comprises an insertion sequence incorporated in a region which can be transferred into a target cell and integrated into a recombinant provirus when said target cell is infected by a retrovirus comprising said sequence of retroelements, said insertion sequence comprising a nucleotide sequence of interest which can be integrated into the genome of a target cell as well as a recognition sequence, preferably a single recognition sequence which can be recognized by a recombinase.

2. Sequence of retroelements according to Claim 1, characterized in that said insertion sequence is incorporated into a cis-acting region of said sequence of retroelements.

3. Sequence of retroelements according to Claim 1, characterized in that said insertion sequence is incorporated into the 3' LTR or 5' LTR region of said sequence of retroelements.

4. Sequence of retroelements according to Claim 1, characterized in that said insertion sequence is incorporated into the U3 region of the 3' LTR, the U5 region of the 5' LTR or the R region of said retroviral sequence.

5. Sequence of retroelements according to any one of the preceding Claims, characterized in that said recognition sequence which can be recognized by a recombinase is situated upstream from said nucleotide sequence of interest.

6. Sequence of retroelements according to any one of the preceding Claims, characterized in that it comprises a nucleotide sequence coding for a recombinase capable of recognizing said recognition sites.

7. Sequence of retroelements according to Claim 6, characterized in that the nucleotide sequence coding for a recombinase is situated between the 5' LTR and 3' LTR regions of said sequence of retroelements.

8. Sequence of retroelements according to Claim 6 or 7, characterized in that the nucleotide sequence coding for a recombinase codes for the Cre protein.

9. Sequence of retroelements according to any one of the preceding Claims, characterized in that said recognition sequence comprises the recognition site LoxP

10. Sequence of retroelements according to any one of the preceding Claims, characterized in that said nucleotide sequence of interest contains a sequence coding for a protein or an RNA of interest, in particular an antisense RNA or a ribozyme sequence.

11. Sequence of retroelements contained in the plasmid deposited on 13 June 1995 with the CNCM under No.I-1599.

12. Retroviral vector characterized in that it comprises a sequence of retroelements according to any one of the preceding Claims.

13. Retroviral vector according to Claim 12, characterized in that said vector is a defective Moloney murine leukemia provirus comprising said recognition site in its U3 region of the 3' LTR, its U5 region of the 5' LTR or its R region.

14. Cell host having integrated in its genome a proviral structure comprising a single LTR sequence of a retroviral vector according to Claim 12 or 13, said LTR sequence comprising a unique copy of a nucleotide sequence of interest.

15. Cell host according to Claim 14, characterized in that it is a eukaryotic cell whose proviral structure is essentially free of PBS sequences, encapsidation and dimerization signal and PPT.

16. Method for introducing a gene into a host cell, said method being characterized in that it comprises the infection of said host cell by the retroviral vector according to Claim 12 or 13.

17. Method for expressing a molecule aided by a sequence of interest in a host cell, said method being characterized in that it comprises :

- the infection of said host cell by a retroviral vector according to Claim 12 or 13 comprising said nucleotide sequence of interest;
- the growth of said host cell under conditions permitting the expression of said nucleotide sequence of interest; and
- the production of the desired molecule.

18. Use of a retroviral sequence according to any one of the Claims 1 to 11 in the production by host cells of proteins or RNAs encoded in a nucleotide sequence of interest.

19. Use of a retroviral vector according to Claim 12 or 13 in the production by host cells of proteins encoded in a nucleotide sequence of interest.

20. Use of a retroviral sequence according to any one of the Claims 1 to 11 in the medical treatment of a patient for the purpose of integrating a nucleotide sequence of interest into the genome of target cells.

21. Use of a retroviral vector according to Claim 12 or 13 in the medical treatment of a patient for the purpose of integrating a nucleotide sequence of interest in the genome of target cells.

22. Pharmaceutical composition containing a retroviral vector according to Claim 12 or 13 in combination with an acceptable pharmaceutical excipient.

23. Use of the composition according to Claim 22 for the purpose of integrating a nucleotide sequence of interest comprising a nucleotide sequence capable of being expressed in the genome of target cells, said composition being constituted by an effective amount of a retroviral vector according to Claim 12 or 13.

24. Pharmaceutical composition constituted by host cells according to Claim 14.

FIG_1a

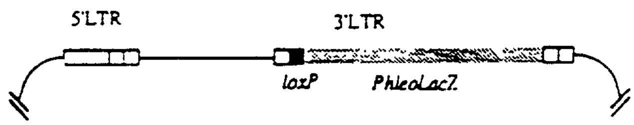


FIG_1b

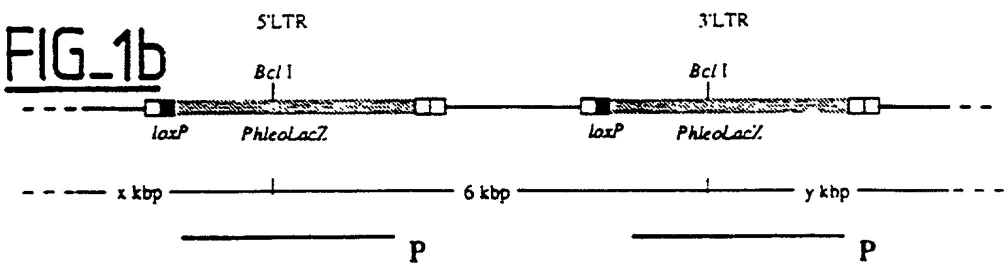


FIG_1c

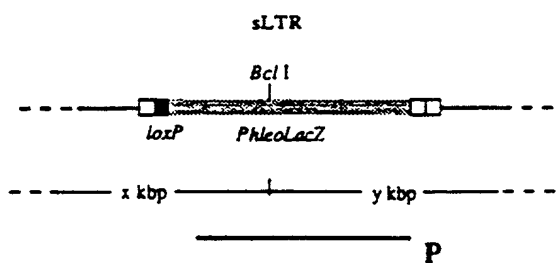


FIG_1d

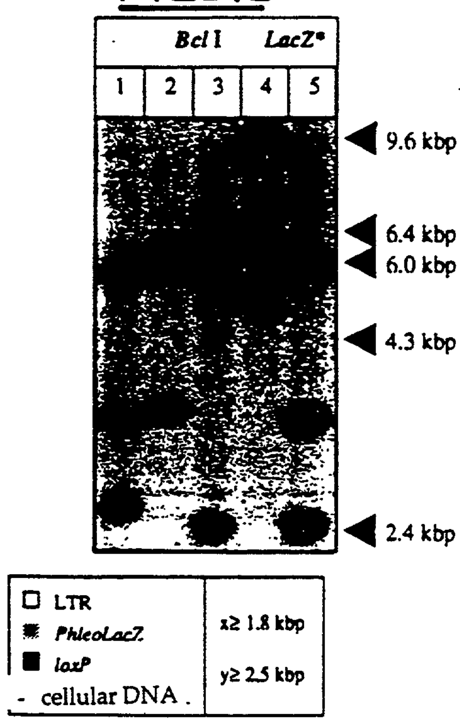


FIG_1e

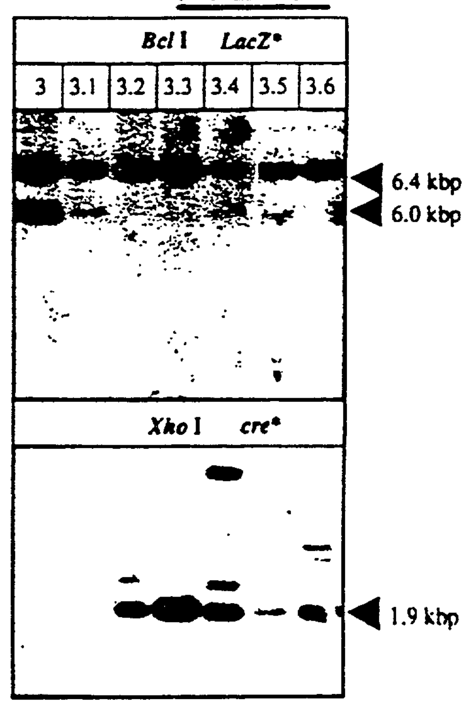


FIG_2a

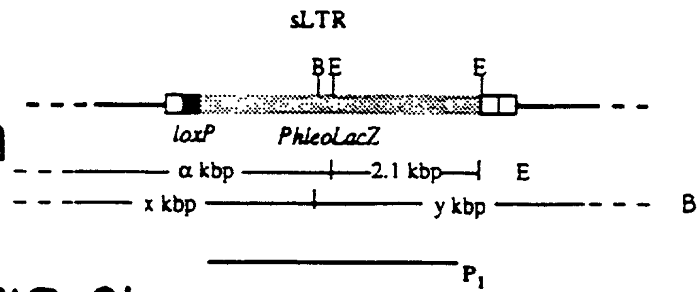


FIG_2b

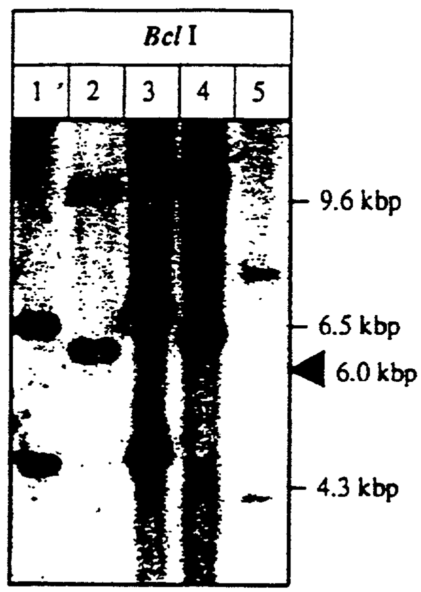
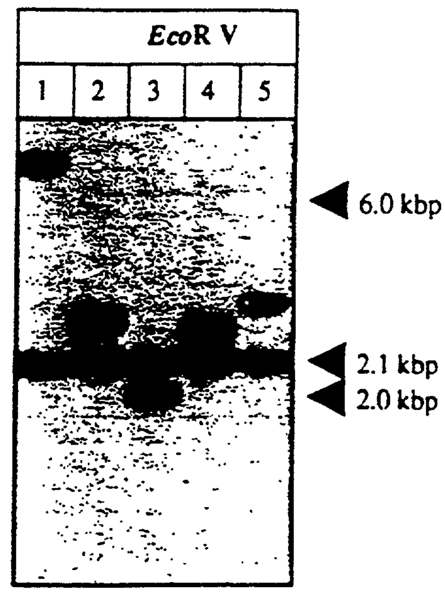


FIG_2c



□ LTR	
▒ *PhleoLacZ*	E : *EcoR* V
■ *loxP*	B : *Bcl* I
— ADN cellulaire	
x≥ 1.8 kbp y≥ 2.5 kbp	α≥ 1.9 kbp

FIG_3a

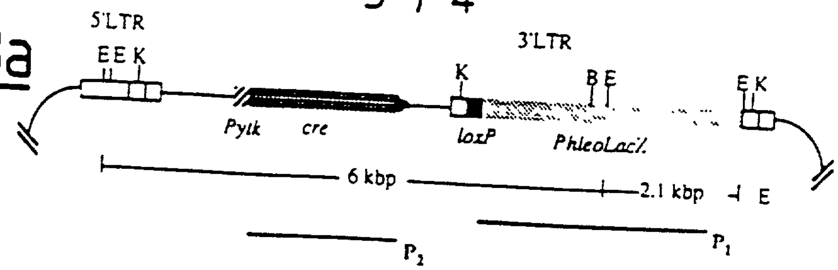


FIG_3b

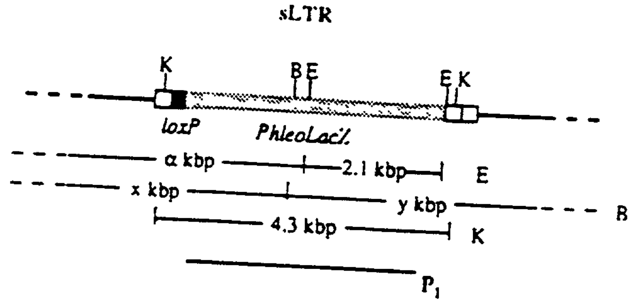


FIG_3c

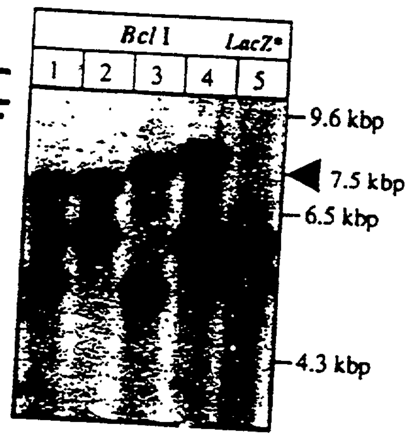


FIG_3d

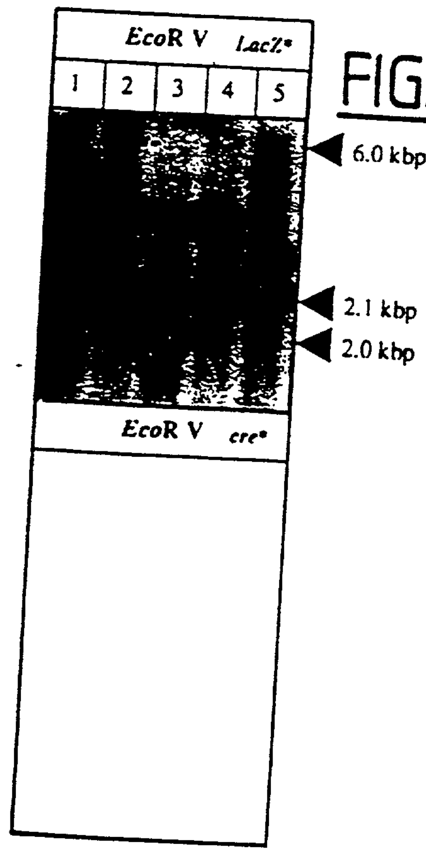
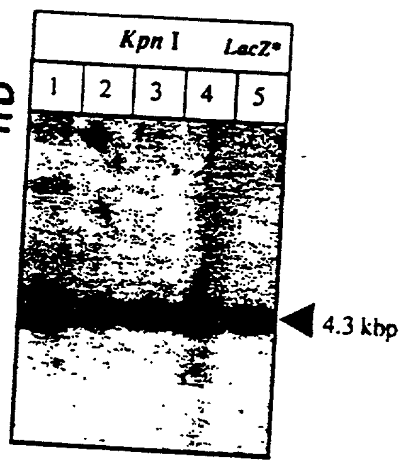


FIG_3e



- LTR
- *PhleoLacZ*
- *loxP*
- *cre*
- Pytk promoter
- cellular DNA

E : *EcoR V*

B : *Bcl I*

K : *Kpn I*

x≥ 1.8 kbp
y≥ 2.5 kbp
α≥ 1.9 kbp

Transfection of the ψ-2 transcomplementing cell line with the plasmid

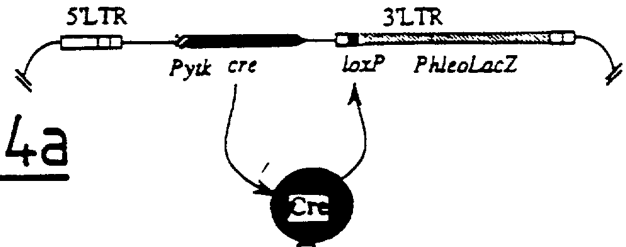


FIG_4a

Infection of the NIH3T3 cells

Integration of the MCreloxPL provirus into the NIH3T3 cell line

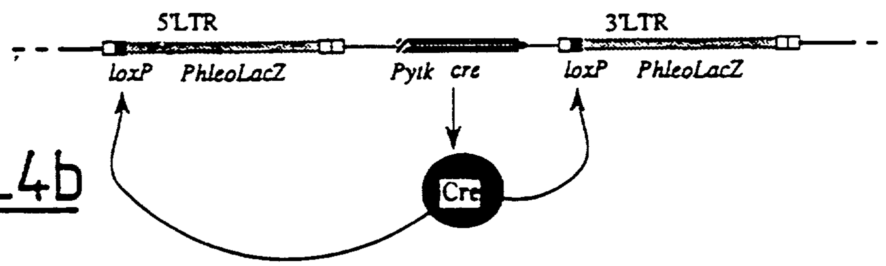


FIG_4b

Recombination of the two loxP sites by the enzyme Cre

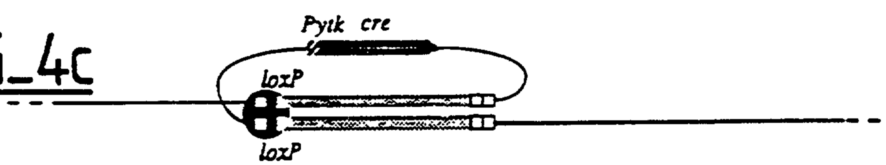


FIG_4c

Recombination

The result of the recombination through the intermediary of Cre-lox is an sLTR

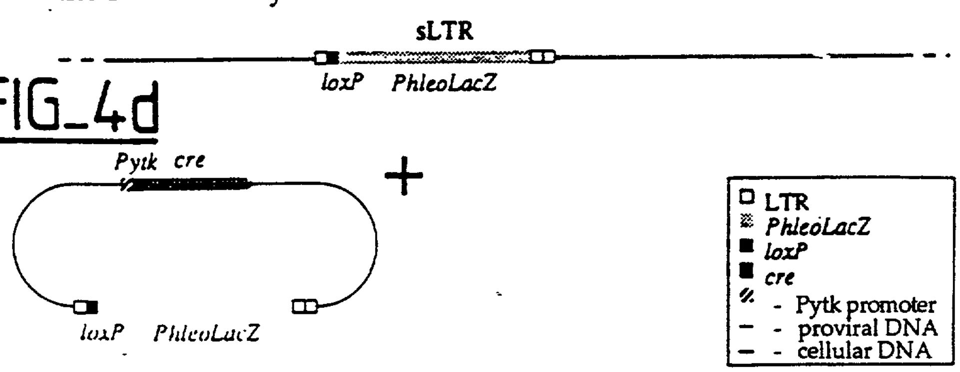


FIG_4d

DZ554r

ATTORNEY DOCKET NO: ____________

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that: my residence, post office address and citizenship are as stated below next to my name; that I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

SEQUENCE OF NATURAL OR SYNTHETIC RETROELEMENTS ENABLING NUCLEOTIDE SEQUENCE INSERTION INTO A EUKARYOTIC CELL.

the specification of which is ☐ attached and/or ☐ was filed on .... as Application Serial No. .... and was amended on (if applicable) ....

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

COUNTRY	APPLICATION NUMBER	DATE OF FILING	PRIORITY CLAIMED UNDER 35 U.S.C. 119
FRANCE	95/09587	07 August 1995	☒ YES ☐ NO
			☐ YES ☐ NO

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

APPLICATION NUMBER	DATE OF FILING	STATUS (Patented, Pending, Abandoned)
PCT/FR 96/01178	July 25, 1996	

I hereby appoint the following attorneys to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: Finnegan, Henderson, Farabow, Garrett and Dunner, Reg. No. 22,540; Douglas B. Henderson, Reg. No. 20,291; Ford. F. Farabow, Jr., Reg. No. 20,630; Arthur S. Garrett, Reg. No. 20,338; Donald R. Dunner, Reg. No. 19,073; Brian G. Brunsvold, Reg. No. 22,593; Tipton D. Jennings, IV, Reg. No. 20,645; Jerry D. Voight, Reg. No. 23,020; Laurence R. Hefter, Reg. No. 20,827; Kenneth E. Payne, Reg. No. 23,098; Herbert H. Mintz, Reg. No. 26,691; C. Larry O'Rourke, Reg. No. 26,014; Albert J. Santorelli, Reg. No. 22,610; Michael C. Elmer, Reg. No. 25,857; Richard H. Smith, Reg. No. 20,609; Stephen L. Peterson, Reg. No. 26,325; John M. Romary, Reg. No. 26,331; Bruce C. Zotter, Reg. No. 27,680; Dennis P. O'Reilley, Reg. No. 27,932; Allen M. Sokal, Reg. No. 26,695; Robert D. Bajefsky, Reg. No. 25,387; Richard L. Stroup, Reg. No. 28,478; David W. Hill, Reg. No. 28,220; Thomas L. Irving, Reg. No. 28,619; Charles E. Lipsey, Reg. No. 28,165; Thomas W. Winland, Reg. No. 27,605; Basil J. Lewris, Reg. No. 28,818; Robert J. Gaybrick, Reg. No. 27,890; Martin I. Fuchs, Reg. No. 28,508; E. Robert Yoches, Reg. No. 30,120; Stephen J. Rosenman, Reg. No. 29,209; Barry W. Graham, Reg. No. 29,924; Thomas H. Jenkins, Reg. No. 30,857; and .... Please address all correspondence to FINNEGAN, HENDERSON, FARABOW, GARRETT AND DUNNER, 1300 I Street, N.W., Washington, D.C. 20005, Telephone No. (202) 408-4000.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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